PB90-215906

Modulating Effect of Body Temperature on the Toxic Response Produced by the Pesticide Chlordimeform in Rats

(U.S.) Health Effects Research Lab., Research Triangle Park, NC

1989

U.S. Department of Commerce
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MS-88-008

TECHNICAL REPORT DATA
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1. REPORT NO. EPA/600/J-89/369	2. PB 90 215906/AS
3. RECIPIENT'S ACCESSION NO.	
4. TITLE AND SUBTITLE MODULATING EFFECT OF BODY TEMPERATURE ON THE TOXIC RESPONSE PRODUCED BY THE PESTICIDE CHLORDIMEFORM IN RATS	5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) William P. Watkinson[1], Jerry W. HIghfill[2] Christopher J. Gordon[3]	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS [1]EPA,HERL,ITD,TB, RTP, NC 27711 [2]EPA,HERL,EEBD,BB, RTP, NC 27711 [3]EPA,HERL,NTD,BTB, RTP, NC 27711	10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research & Development Health Effects Research Laboratory - RTP, NC U.S. Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/10

15. SUPPLEMENTARY NOTES Project Officer - W. P. Watkinson
~~Published in Fund. Appl. Toxicol.~~
Journal of Toxicology and Environmental Health; Vol. 28; pp. 483-500; 12/89

16. ABSTRACT
Previous studies from this laboratory have demonstrated significant deficits in cardiovascular function in rats exposed to the pesticide chlordimeform (CDM) when body core temperature (Tco) was maintained at 37°C. To investigate the role of Tco on CDM toxicity, similar experiments were conducted over a range of Tco's. Adult rats (N= 30) were anesthetized with sodium pentobarbital (35 mg/kg) and randomly assigned to one of six equal groups. Groups were paired and Tco was maintained in the rats in each of the respective group pairs at one of three levels (37°, 35°, or 33°C). Rats in one group at each temperature level (Groups T37, T35, and T33) were injected intraperitoneally with 60 mg/kg of CDM. Animals in the corresponding temperature-matched groups (Groups C37, C35, and C33, respectively) received volume-matched injections of normal saline vehicle and served as time-paired controls. The electrocardiogram and heart rate (HR) were monitored throughout the experimental procedure. There was a significant decrease in HR in all CDM-treated groups when compared to the control group animals. The magnitude of the observed cardiac effect was attenuated in the T35 group when compared to that of the other treated groups. Similarly, lethality rates (# deaths/# total) for the T37, T35, and T33 groups were 2/5, 0/5, and 3/5, respectively; there were no deaths among the control group animals. From these and previous data from this laboratory, we conclude there may be a beneficial effect of hypothermia in rats exposed to toxic agents which is maximal around 35°C.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 20
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE A03

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

PB90-215906
EPA/600/J-89/369

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MODULATING EFFECT OF BODY TEMPERATURE ON THE TOXIC RESPONSE PRODUCED BY THE PESTICIDE CHLORDIMEFORM IN RATS

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Previous studies from this laboratory have demonstrated significant deficits in cardiovascular function in rats exposed to the pesticide chlordimeform (CDM) when body core temperature (T_{co}) was maintained at 37°C. To investigate the role of T_{co} on CDM toxicity, similar experiments were conducted over a range of T_{co} values. Adult rats (n = 30) were anesthetized with sodium pentobarbital (35 mg/kg) and randomly assigned to one of six equal groups. Groups were paired and T_{co} was maintained in the rats in each of the respective group pairs at one of three levels (37, 35, or 33°C). Rats in one group at each temperature level (groups T37, T35, and T33) were injected intraperitoneally with 60 mg/kg of CDM. Animals in the corresponding temperature-matched groups (groups C37, C35, and C33, respectively) received volume-matched injections of normal saline vehicle and served as time-paired controls. The electrocardiogram and heart rate (HR) were monitored throughout the experimental procedure. There was a significant decrease in HR in all CDM-treated groups when compared to the control group animals. The magnitude of the observed cardiac effect was attenu-

This manuscript has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Portions of this research were presented at the fall meeting of the American Physiological Society in New Orleans, La., October 5–9, 1986.

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ated in the T35 group when compared to that of the other treated groups. Similarly, lethality rates (number of deaths/total) for the T37, T35, and T33 groups were 2/5, 0/5, and 3/5, respectively; there were no deaths among the control-group animals. From these and previous data from this laboratory, we conclude there may be a beneficial effect of moderate hypothermia in rats exposed to toxic agents while more severe hypothermia appears to offer no advantage and may actually exacerbate the toxic effect.

INTRODUCTION

Chlordimeform [*N'*-(4-chloro-*o*-tolyl)-*N,N*-dimethylformamidine, Galecron, Fundal, CDM], which has recently undergone a voluntary ban in the United States, is a prototypical pesticide of the formamidine group that has been used primarily to control ticks and mites in cattle and as a pesticide on cotton (Kurtz et al., 1987). Although the incidence of reported adverse toxic effects in humans has been limited (Hayes, 1982; Kimbrough, 1980; Kurtz et al., 1987), a number of laboratory investigations have clearly demonstrated the toxicity of CDM in mammals (Benezet et al., 1978; Lund et al., 1979; Matsumura and Beeman, 1976). Reported toxic effects have included physiological and behavioral perturbations, as well as acute lethality following exposure to CDM (Boyes et al., 1985; Chinn et al., 1977; Gordon et al., 1985; Olson et al., 1978; Robinson and Smith, 1977). While the mechanism(s) of action of CDM remains unclear, there is a general consensus that the cardiovascular system represents a principal target organ system for CDM toxicity (Lund et al., 1978a; Rieger et al., 1981). Cardiac and/or respiratory collapse has been implicated as the cause of death following acute exposure to CDM (Lund et al., 1978b; Pfister et al., 1978). Chlordimeform also exerts profound inhibition on monoamine oxidase activity (Beeman and Matsumura, 1973).

A series of studies (Watkinson, 1985, 1986a, 1986b) from this laboratory using the anesthetized rat in which body core temperature (T_{co}) was maintained at 37°C has shown that the toxic response to CDM on the cardiovascular system can be functionally divided into two distinct components. The acute portion of the response is characterized by a striking decrease in heart rate (HR), blood pressure (BP), and respiratory rate, accompanied by significant quantitative and arrhythmic changes in electrocardiographic (ECG) waveform parameters. These acute effects are dose-related, rapid in onset, and exhibit apparent recovery within 3–5 min. In contrast, the delayed portion of the toxic response, which occurs approximately 10–15 min following administration of CDM, is characterized by abrupt decreases in the steady-state HR and BP while ECG parameters remain essentially unchanged. The delayed effects are persistent and do not exhibit a dose-response relationship.

A more recent study from this laboratory (Watkinson and Gordon, 1987) has shown that CDM administration in the unanesthetized, non-

temperature-maintained rat produces HR and ECG effects that are similar in character to the delayed effects of the previous studies but of lesser magnitude and duration. In the latter study, exposure to CDM also induced a nearly 2°C decrease in T_{co}.

The primary methodological differences between the above studies involved the use of anesthesia and the maintenance of body temperature. From these studies alone, it was not possible to distinguish between the relative contribution of either of these confounding variables. The present study was designed to investigate the specific role of T_{co} on CDM toxicity. The results of this study, when combined with previous findings, permit a more accurate assessment of the effects of anesthesia per se and the effects of changes in body temperature on the toxic response produced by this pesticide. Furthermore, these results suggest a potential beneficial role for the moderate hypothermia observed following exposure to a variety of toxic agents.

METHODS

Animals

Adult, male Sprague-Dawley rats (n = 30) were used in these studies. Animals were obtained (Charles River Breeding Laboratory, Kingston, N.Y.) at 50 d of age and housed in plastic cages on pine shaving bedding in a temperature-controlled (22–26°C) and light-controlled (12 h light : 12 h dark), biologically clean room until studied. They were allowed access to tap water and laboratory feed ad libitum. Animals were studied at 55–72 d of age and ranged in weight from 286 to 404 g.

Experimental Preparation

Animals were lightly anesthetized with methoxyflurane (Metafane, Pittman-Moore Company), weighed, and more deeply anesthetized with sodium pentobarbital (35 mg/kg, ip). Animals were then placed in a specially constructed temperature-controlling apparatus (Fig. 1) designed to stabilize T_{co} around a selected level. Briefly, animals were placed in a supine position on the surface of a water-filled Plexiglas trough. A water bath (rnodel FE; Haake, Inc.) was used to continually circulate water through the trough. The water temperature was maintained approximately 5°C below the selected T_{co} level. The top surface of the trough was V-shaped to permit maximum heat transfer capability. A thermistor temperature probe (model 402; Yellow Springs Instrument Co.) was inserted approximately 6 cm past the anal sphincter. The output of the temperature probe was connected to a temperature controller (model 74; Yellow Springs Instrument Co.), which was set to the selected T_{co}. An infrared heat lamp and a small fan were connected to the heating and

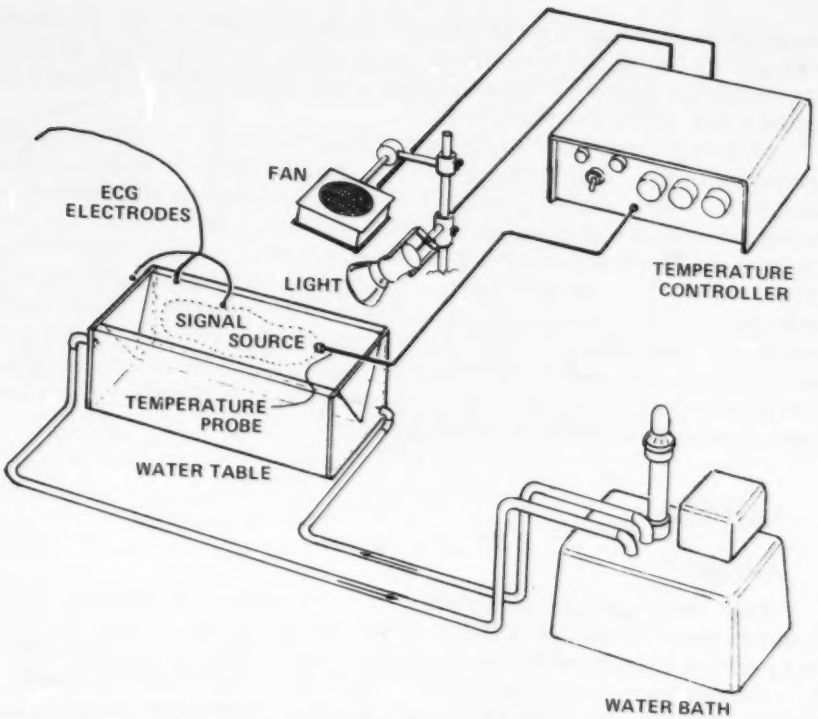

FIGURE 1. Diagram of temperature-controlling apparatus illustrating component interconnections. Apparatus was designed to stabilize body core temperature around selected level. See text for detailed description of use.

cooling outputs, respectively, of the temperature controller. Thus, decreases in T_{co} below the selected temperature level activated the infrared lamp, which warmed the animal, while increases in T_{co} above the selected level served to activate the fan, which cooled the animal. Using this apparatus, T_{co} was maintained within $\pm 0.1°C$ of the desired set point.

Electrodes (model E2; Grass Instrument Company) were implanted subcutaneously on the ventral surface at the junctures of the extremities to monitor the ECG. HR was derived from the ECG signal. All data were monitored on an eight-channel chart recorder (model 7758B; Hewlett Packard Company) and recorded on magnetic tape using a four-channel FM cassette recorder (model R-61; TEAC Corporation), as previously described (Watkinson, 1985).

A Teflon catheter (model 22G1; Becton, Dickinson and Company) was inserted into the abdominal cavity until the tip of the catheter was positioned approximately 2 cm into the peritoneal space. A 1-ml injection

syringe was attached to the catheter for intraperitoneal (ip) injection of the test compounds.

Experimental Procedure

Animals (n = 30) were randomly assigned to one of six equal groups, three of which received CDM treatment while the remaining three served as vehicle controls. Treated and control groups were paired, and T_{co} was maintained in the rats in each of the respective group pairs at one of three levels. The three temperature levels at which the animals were maintained were chosen to correspond to conditions of normothermia, moderate hypothermia, and severe hypothermia. Thus, the six dose groups consisted of animals maintained at either 37, 35, or 33°C and injected either with a single dose of CDM (groups T37, T35, and T33, respectively) or with vehicle only (groups C37, C35, and C33, respectively). After the initial preparatory procedures, animals were allowed to stabilize at the selected T_{co}. While it generally took longer for the animals assigned to the lower temperature groups to reach the desired T_{co}, all animals were stable at the selected T_{co} for at least 20 min prior to beginning the experiment. The experimental time period commenced (time = 0) with the injection of the test compound exactly 60 min after induction of anesthesia in all animals and ran continuously for an additional 60 min.

Electrocardiograms were monitored throughout the experiment and HRs were derived from the ECG tracings at selected time intervals. Steady-state HR values were obtained from each animal at approximately 10, 5, and 1 min prior to the beginning of the experimental time period. These three HR values were averaged to obtain an initial preinjection control value. Animals in the treatment groups were then injected via the abdominal catheter with a single dose of 60 mg/kg CDM (chlordimeform HCl, 98% pure; Chem Service, Westchester, Pa.) dissolved in normal saline. All concentrations were adjusted so that the volumes administered were equivalent to 1 μl/g body weight. Animals in the control groups received a similar volume of vehicle only. In addition to preinjection recordings, HR analyses were conducted at 5-min intervals throughout the 60-min experimental time period. ECGs were qualitatively examined for arrhythmias, and all lethalities were noted.

Statistical Analysis

Statistical analyses were conducted using the general linear models procedure of the Statistical Analysis System (SAS Institute, Inc., 1982). A multivariate repeated-measures analysis was used to test for differences between groups. Probability values less than .05, were considered significant.

RESULTS

The effects observed following a single ip injection of 60 mg/kg CDM in the T37 group animals were comparable to those reported previously for temperature-maintained animals undergoing a similar experimental procedure (Watkinson, 1986b). Due to the nature of the ip exposure, there was no discernible acute cardiotoxic response pattern; however, the observed effects were essentially equivalent to those of the previously described delayed portion of the toxic response. These effects were primarily characterized by significant decreases in HR in the absence of qualitative changes in th ECG waveform. In addition, exposure to CDM at a T_{co} of 37°C produced a moderate degree of lethality among the treated animals. The toxic effects seen in the T33 group animals were comparable in magnitude to those of the T37 group animals. The effects observed in the T35 group animals, however, were attenuated when compared to those of the other CDM-treated group animals.

Heart Rate Response

There was a significant decrease in HR in all treated groups following exposure to CDM when compared to their respective temperature-matched control groups (Table 1). These decreases, which were apparent as early as 5 min postinjection and reached their nadir at 15 min postinjection, ranged from a decrease of 66 beats per minute (bpm) in the T33 group animals to a decrease of 86 bpm in the T37 group animals. The positive correlation observed between T_{co} and the magnitude of the decrease in HR was primarily a function of the higher preinjection HRs of the animals maintained at the higher body temperatures. There were no decreases in HR in the vehicle-treated group animals.

To facilitate comparisons among groups, the HR values for each animal were normalized with respect to the animal's initial preinjection control value, and the resulting data are shown graphically in Fig. 2. While HR for the C37 group animals remained constant for the entire experimental time period, HR values for the T37 group decreased ~24% within 15 min and remained depressed. The HR values of the C33 group animals rose gradually for the first 30 min of the observation period and rose somewhat more rapidly during the latter portion of the experiment. Initially, the HR response observed in the T33 group animals following injection of CDM was similar to that of the T37 group animals, decreasing ~22% over the first 15 min postinjection. In contrast to the persistent decrease observed in the T37 group animals, however, HR in the T33 group animals rose gradually over the latter portion of the experiment. Despite this subsequent rise in relative HR, the final HR values observed in the T33 group animals were still lower than the initial preinjection control values and significantly lower than the time-matched control values of the C33 group animals. The overall HR response of the C35 group

TABLE 1. Heart Rate for Intraperitoneal Vehicle- and Chlordimeform-Treated Temperature-Maintained Adult Rats[a]

Group	Preinjection	Postinjection											
		5[b]	10	15	20	25	30	35	40	45	50	55	60
Vehicle, 37°C	324 ± 7	328 ± 8	326 ± 9	325 ± 8	324 ± 8	324 ± 7	324 ± 8	324 ± 8	324 ± 7	320 ± 8	320 ± 8	322 ± 9	323 ± 8
Vehicle, 35°C	249 ± 9	253 ± 9	251 ± 9	257 ± 8	253 ± 8	256 ± 9	258 ± 10	283 ± 20	299 ± 29	295 ± 27	302 ± 23	308 ± 24	313 ± 24
Vehicle, 33°C	228 ± 7	234 ± 7	234 ± 7	234 ± 5	237 ± 5	242 ± 3	248 ± 5	253 ± 8	257 ± 10	269 ± 12	272 ± 14	275 ± 15	295 ± 26
Chlordimeform, 37°C	364 ± 9	284 ± 9	279 ± 14	278 ± 13	280 ± 10	284 ± 10	285 ± 11	289 ± 12	291 ± 12	293 ± 13	292 ± 13	295 ± 13	296 ± 9
Chlordimeform, 35°C	277 ± 11	233 ± 4	234 ± 8	228 ± 9	237 ± 8	241 ± 10	245 ± 11	248 ± 12	249 ± 12	254 ± 12	252 ± 12	254 ± 13	258 ± 12
Chlordimeform, 33°C	269 ± 17	217 ± 6	205 ± 18	203 ± 24	205 ± 19	210 ± 17	218 ± 16	229 ± 14	235 ± 13	238 ± 12	235 ± 16	238 ± 12	245 ± 10

[a]Each value represents mean ± SEM for five animals.
[b]Time (min) postinjection.

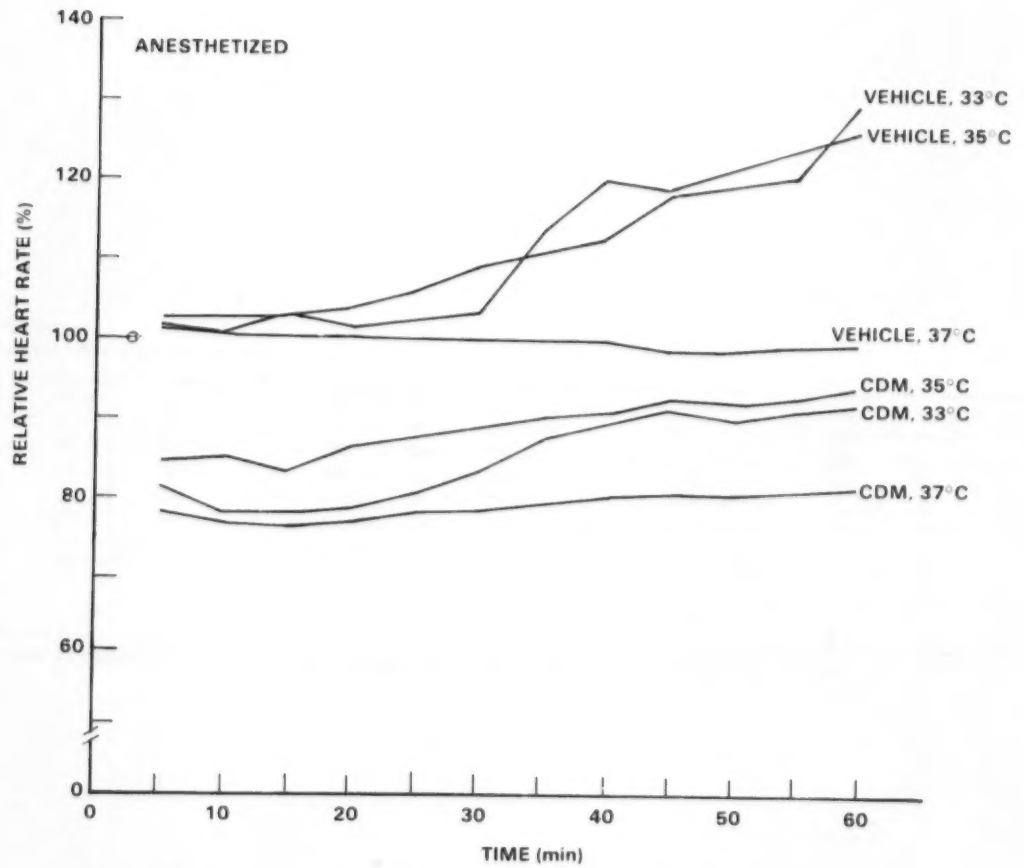

FIGURE 2. Relative HR response (mean; $n = 5$) of anesthetized, adult rats to single intraperitoneal injection of 60 mg/kg CDM or normal saline vehicle. HR data were first normalized with respect to preinjection values. Circle with bar (⊖) indicates initial control value for all groups. Body temperature of animals within groups was maintained within ±0.1°C of selected temperature using specially designed temperature-controlling apparatus. All HR values of treated animals were significantly different from time-paired HR values of control animals ($p \leq .05$).

animals was similar to that of the C33 group animals, with the final HR values for these two vehicle-treated control groups being 126 and 129%, respectively, of their initial preinjection control values. The response pattern seen in the C35 group animals appeared to be more biphasic in nature than that of the C33 group animals, exhibiting a very slight elevation over the initial portion of the experimental time period and a more rapid rise during the latter portion of the experiment. The HR response shown by the T35 group animals, while significantly depressed when compared to the initial preinjection control value and to the HR value of the time-matched control group animals, was attenuated when compared to the response of the other treated-group animals. The HR of the T35 group animals rose over the latter portion of the experimental time period, an observation consistent with the response of the other group animals maintained at subnormal T_{co} levels.

Electrocardiographic Response

The ECG waveforms of treated animals were qualitatively similar to those of their temperature-matched controls. The few arrhythmias observed during the study were predominantly premature atrial and premature ventricular contractions. Again, these results were consistent with the delayed toxic response pattern seen in previous studies.

Lethality

Five of the 15 treated animals died following exposure to CDM. Of these deaths, two occurred among the T37 group animals and three occurred among the T33 group animals. These data are summarized in Fig. 3. Most of these deaths occurred at or shortly after the end of the 60-min experimental time period and appeared to result from cardiovascular collapse. There were no deaths among the vehicle-treated animals.

DISCUSSION

A series of earlier studies from this laboratory (Watkinson, 1985, 1986a, 1986b) raised interesting questions regarding the interactions of anesthesia, body temperature, and physiological function in mammals in response to exposure to toxic agents. General anesthesia may provide several opportunities for modulation of the observed response to a toxic agent. The direct biochemical interaction of anesthetic and toxic agents could produce additive effects. Such anesthetic-like actions/interactions have been proposed with respect to CDM (Lund et al., 1978a; Pfister et al., 1978). Modulation of the toxic response could involve a common pathway resulting from exposure to both anesthesia and toxic agents. Both sodium pentobarbital and CDM have been reported to affect T_{co}

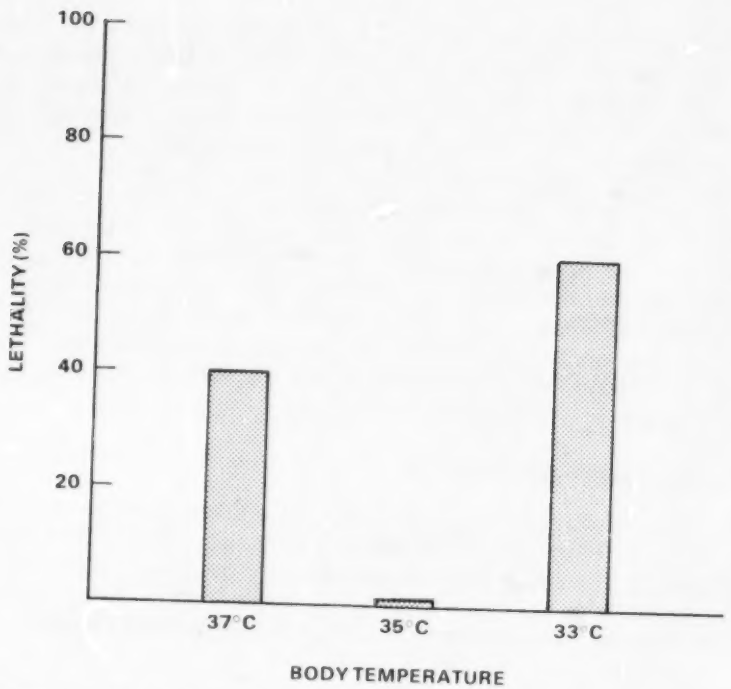

FIGURE 3. Percent lethality observed following single intraperitoneal injection of 60 mg/kg CDM in anesthetized, adult rats (n = 5). Body temperature of animals within groups was maintained within ±0.1°C of selected temperature using specially designed temperature-controlling apparatus. There were no deaths observed in control-group animals.

and such changes in T_{co} have been shown to alter physiological functional responses. Finally, it is well known that anesthetics such as sodium pentobarbital significantly alter the functional response of such major organ systems as the cardiovascular, respiratory, and thermoregulatory systems (Harvey, 1975). Such changes in systemic function may in turn independently influence the observed toxic effect. The assessment of the relative contribution of these interactive variables toward the observed toxic response presents a difficult problem in the interpretation of toxicological studies.

Earlier studies from this laboratory (Watkinson, 1985, 1986a, 1986b) demonstrated consistent, significant toxic effects in anesthetized, temperature-maintained (T_{co} = 37°C) rats following exposure to CDM. It was particularly noteworthy, however, that the magnitude and duration of the observed effects were greater than those described previously by other laboratories (Lund et al., 1978a; Matsumura and Beeman, 1976; Rieger et al., 1981) where T_{co} was presumably not maintained. A subse-

quent study from this laboratory (Watkinson and Gordon, 1987) using unanesthetized rats yielded results more in line with those reported previously by other investigators. However, interpretation of the results in the follow-up study was complicated by the observation that CDM also induced a nearly 2°C decrease in T_{co}. It was proposed that the dichotomy between the toxic effects reported in our studies and those from other laboratories was most likely attributable to the differences in T_{co} of the animal models rather than to a direct effect of sodium pentobarbital. The artificial maintenance of body temperature during our experimental procedures in anesthetized animals appeared to exacerbate the toxicity of CDM when compared to the results of other studies. Alternatively, the ability of the unanesthetized animals to lower T_{co} in response to exposure to CDM seemed to attenuate the toxic response and enhance survival. Despite the inferences gained from these data, it was not possible at that time to exclude the potential contribution of a direct interaction between sodium pentobarbital and CDM to the observed toxic effects. Therefore, the present study was designed and conducted to more fully differentiate between the role of anesthetics per se and the general contribution of changes in T_{co} to the overall toxic response observed following exposure to CDM. A moderately toxic dose was selected in order to produce significant physiological responses and permit the simultaneous monitoring of lethality, an important toxicological end point. The use of the temperature-clamping methodology described above allowed direct comparison of toxic effects over a range of T_{co} values in anesthetized animals. The T_{co} values utilized were chosen to simulate conditions of normothermia, moderate hypothermia, and severe hypothermia in the rat.

The results of the present study support the hypothesis that the magnitude of the toxic effect of CDM is primarily modulated by changes in T_{co}. There were clear differences in the percent decrease in HR following exposure to CDM at different levels of T_{co}, despite a constant level of anesthesia. The toxic effect was diminished at the T_{co} corresponding to moderate hypothermia; more severe hypothermia appeared to potentiate the toxic response. The lethality data exhibited a similar trend and provide additional evidence in support of this conclusion.

Comparison of the data from the present study with those from previous studies from this laboratory that utilized similar experimental protocols further strengthens the above conclusion. For example, the HR response following injection of 60 mg/kg CDM in the T37 group animals in the present study can be compared with that reported previously (Watkinson, 1986b) in a study using anesthetized animals whose T_{co} was also maintained at or slightly above 37°C (Fig. 4*a* and *b*). In the present study, HR dropped 20–25% and remained depressed for at least 60 min. The previous study reported a 25–30% decrease in HR, which was also maintained for at least 60 min. Lethality in the T37 group animals in the

present study was 40% (2 deaths/5 animals), while 37.5% (3 deaths/8 animals) of the animals in the previous study died.

In the present study, HR in the T35 group animals initially decreased 15–20%. HR tended to rise toward the end of the experimental time period and at 60 min postinjection was only depressed 7% compared to the

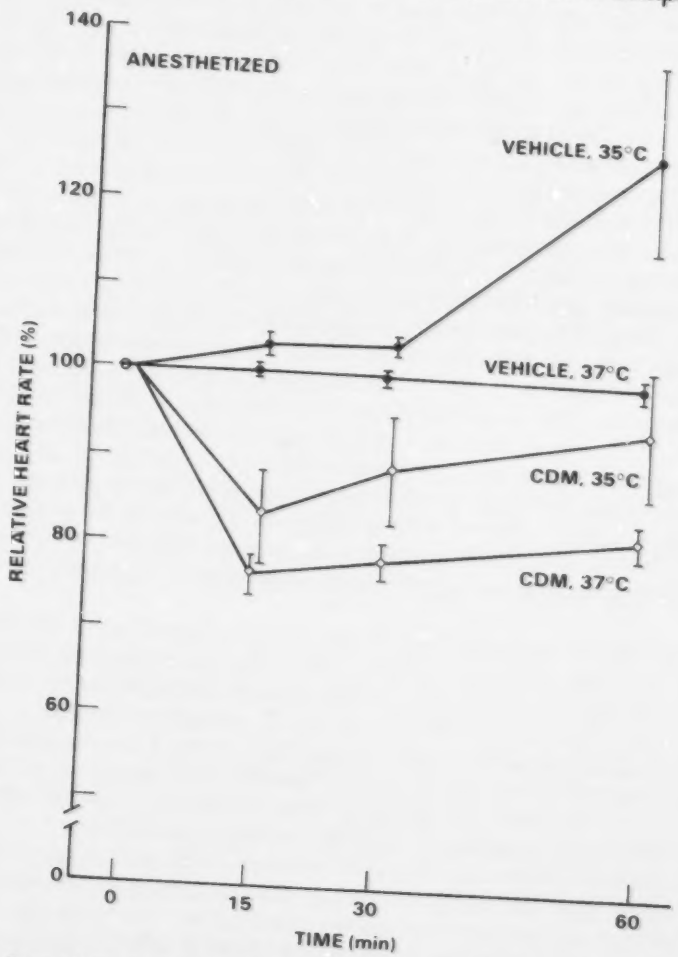

FIGURE 4. Comparison of relative HR response following exposure to CDM under different experimental conditions. All animals received a single intraperitoneal injection of 60 mg/kg CDM or normal saline vehicle. HR data have been normalized with respect to preinjection values. Circles with bar (⊙) indicate initial control value for all groups. (a) Adult rats; n = 5/group; anesthetized; temperature-maintained (redrawn from present study). With the exception of the relative HR response at time = 60 min in (c), all HR values of treated animals were significantly different from time-paired HR values of control animals ($p \leq .05$).

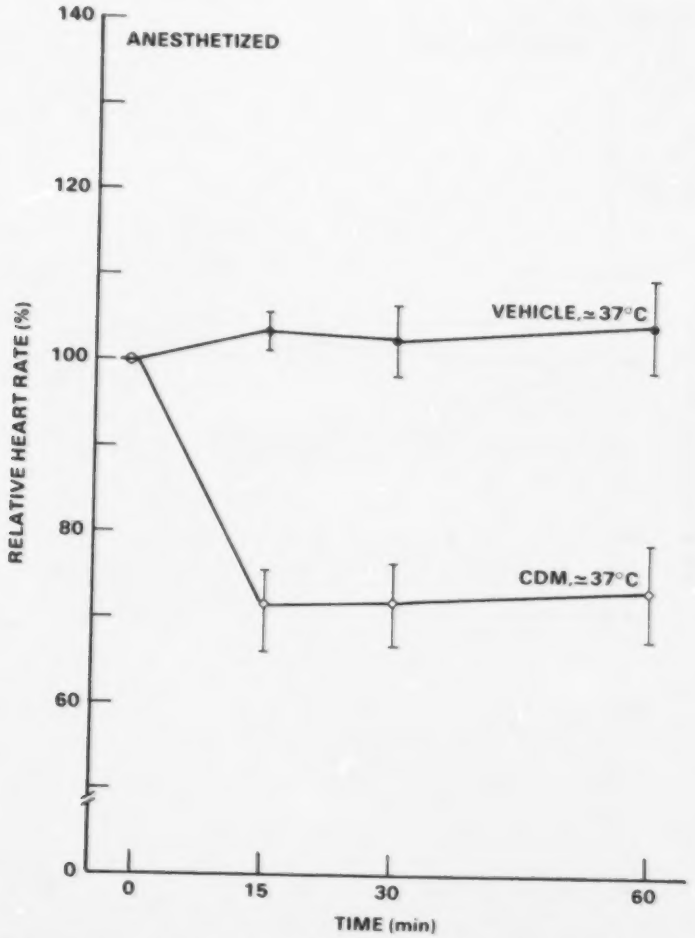

FIGURE 4. Comparison of relative HR response following exposure to CDM under different experimental conditions. All animals received a single intraperitoneal injection of 60 mg/kg CDM or normal saline vehicle. HR data have been normalized with respect to preinjection values. Circles with bar (⊙) indicate initial control value for all groups (*Continued*). (*b*) Postweanling rats; n = 8/group; anesthetized; temperature-maintained (modified from Watkinson, 1986b). With the exception of the relative HR response at time = 60 min in (c), all HR values of treated animals were significantly different from time-paired HR values of control animals ($p \leq .05$).

initial control value. In a previous study (Watkinson and Gordon, 1987) using unanesthetized animals injected with 60 mg/kg CDM and maintained at an ambient temperature of approximately 22°C, T_{co} was observed to decrease an average of 1.6°C at 60 min postinjection. In conjunction with this decrease in T_{co}, the observed decrease in HR was

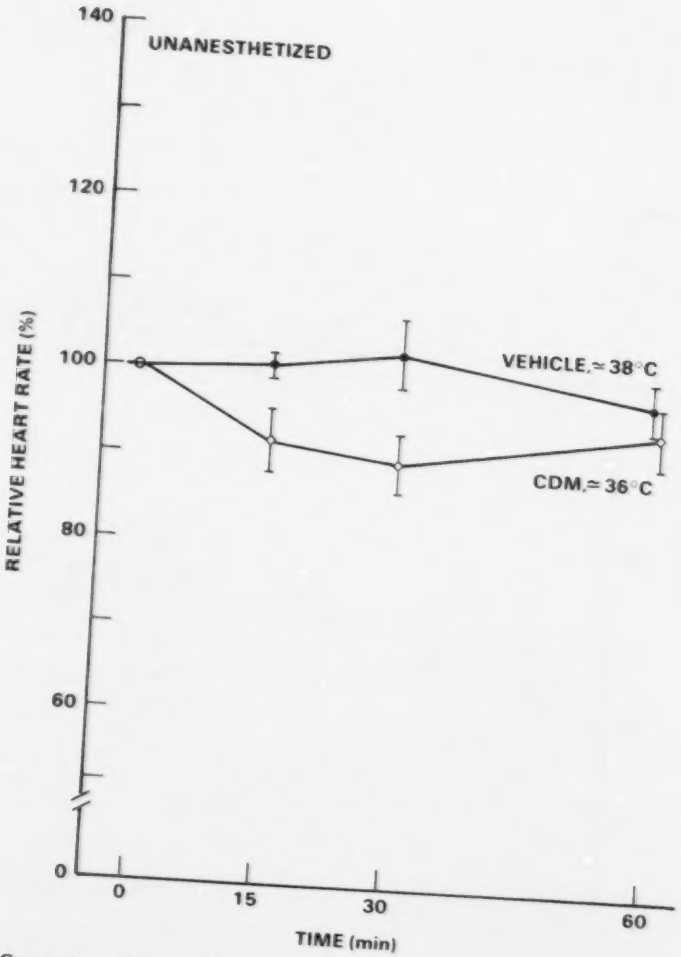

FIGURE 4. Comparison of relative HR response following exposure to CDM under different experimental conditions. All animals received a single intraperitoneal injection of 60 mg/kg CDM or normal saline vehicle. HR data have been normalized with respect to preinjection values. Circles with bar (⊙) indicate initial control value for all groups (*Continued*). (c) Adult rats; $n = 7$/group; unanesthetized; non-temperature-maintained (modified from Watkinson and Gordon, 1987). With the exception of the relative HR response at time = 60 min in (c), all HR values of treated animals were significantly different from time-paired HR values of control animals ($p \leq .05$).

noticeably attenuated. HR initially decreased 10–12% and at 60 min postinjection was 7% below the initial control value. There was no lethality observed in either of these groups of animals. It is clear that the above results from moderately hypothermic animals represent a significant diminution of the toxic response from that observed in animals main-

tained at 37°C, despite the presence or absence of anesthesia. These data appear to confirm the previously proposed protective effect of moderate hypothermia in response to exposure to toxic agents.

It is interesting to note that CDM is a potent inhibitor of monoamine oxidase in both liver and central nervous system and thus would be expected to elicit sympathomimetic properties (Aziz and Knowles, 1973; Bailey et al., 1982; Beeman and Matsumura, 1973). However, the hypotensive and bradycardic effects of CDM observed in the rat in the present and past investigations (Gordon and Watkinson, 1988; Rieger et al., 1981; Watkinson, 1985, 1986a, 1986b) are opposite to that of a sympathetically-mediated response. Thus, these cardiodepressant effects confound the postulated biochemical mechanisms of CDM toxicity.

While the biological rationale for the protective effect of moderate hypothermia is unclear (Gordon et al., 1988), there are several possible explanations. For example, it is known that the rate of a chemical reaction varies directly with the temperature (Q_{10} effect) and that most toxic effects involve chemical reactions. Thus, the degree of toxicity observed following exposure to a xenobiotic agent will be diminished as the rates of chemical reactions responsible for the toxic effects are decreased. A related explanation is based on the observation that hypothermia is associated with a general decrease in metabolic rate. This decrease in metabolic rate would also slow the rate of conversion of xenobiotic agents to their primary metabolites. The relative toxicities of the breakdown products of most toxic agents are unknown; however, in some cases the metabolites of an agent (e.g., CDM) have been reported to be more potent than the agent itself (Benezet et al., 1978; Hayes, 1982).

The rationale underlying the nonlinear and apparently inconsistent nature of the observed temperature modulation response is also unclear. It is somewhat surprising that potentiation was observed to occur at only 4°C below normal T_{co} in the rat; however, the general "U-shaped" pattern of the lethality response curve has been reported previously (Weighe, 1973; Gordon et al., 1988). The combination of the substantial decrease in body temperature along with the concomitant exposure to the toxic agent may have compromised the animal to the point that it was not able to recover. Although other studies from our laboratory have successfully maintained rats at much lower body temperatures using these procedures (unpublished observations), the temperature-clamping methodology has never previously been used in conjunction with exposure to toxic agents.

A second puzzling aspect of the present study concerns the variability of the heart rate response at 60 min postinjection in both the C35 and T35 group animals. This variability is probably attributable to the interplay of several of the experimental procedures utilized and is best explained by examining the experimental protocol. The restraint required by the temperature-clamping methodology is stress-inducing and can

only be used with anesthetized animals. In the present study, the rats were anesthetized for 60 min prior to injection of CDM. Thus, at 30 min postinjection, they had been anesthetized for a total of 90 min and the level of anesthesia was beginning to decrease. As the level of anesthesia diminished, the stress of the temperature-clamping procedure was unmasked. This additional stress was reflected as an increase in heart rate and heart rate variability. It is reasonable to expect that the imposition of subnormal body temperatures would greatly potentiate this stress. Therefore, while the observed response was relatively minor in animals whose body temperatures were maintained at 37°C, it was most apparent in animals whose body temperatures were clamped at subnormal set points. As additional evidence in support of this hypothesis, it should be noted that a similar response was seen for the C33 and T33 group animals (Fig. 2; variability not shown).

The observed decrease in T_{co} in response to exposure to CDM may be more ubiquitous than unique. This response has been observed in our laboratory across a wide variety of toxic agents and exposure regimens. Examples of toxic agents studied have included pesticides (CDM, sulfolane), heavy metals (nickel, tin), ethanol, ozone, and urea, while exposure regimens have included intravenous, intraperitoneal, and inhalation routes of administration. For most of the above toxic agents, animals were exposed to doses ranging from 40 to 60% of the LD50 in order to ensure induction of significant toxic effects without compromising the ability of the unanesthetized animal to select its preferred ambient temperature. In each case, the observed decrease in T_{co} was relatively moderate (~2°C) and appeared to be correlated with a diminished toxic response and/or enhanced survivability (Gordon et al., 1988).

In summary, the results of the present study appear to explain the discrepancies between previous studies from this laboratory and other laboratories. Thus, at a given dose of CDM, the magnitude and duration of the observed toxic effect are primarily a function of T_{co} and appear relatively independent of a direct interaction with sodium pentobarbital. Furthermore, the results of this study support our earlier hypothesis that a slight (~2°C) decrease in T_{co} appears to attenuate the adverse response to CDM and enhance the ability of the animal to survive while a more severe decrease in T_{co} appears to potentiate the toxic response. The similarities between the results of the present study using CDM and the results of other studies from this laboratory using a variety of toxic agents indicate this may be a ubiquitous response—that is, the animal's response to many toxic agents may be to lower its T_{co}. Consequently, the ability to experimentally monitor and control core temperature should be an important consideration for future toxicological studies.

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Received November 14, 1988
Accepted August 8, 1989